ADENOVIRUS CHROMATOGRAPHIC PURIFICATION PROTOCOL

This protocol is adapted from BD Bio Sciences-Clonetech Protocol by the Gene Expression Lab.
This protocol is for use with BD Bio Sciences-Clonetech Systems. For additional technical inquiries, contact Technical Service at 800-662-2566 *or www.clonetech.com*

ADENOVIRUS AMPLIFICATION
FILTER PURIFICATION
PURIFICATION
VIRUS ELUTION
VIRUS ESTIMATION

BEFORE STARTING THE EXPERIMENT

- Pre-warm all media to RT before use
- Prepare Solutions:
  1) Prepare 1X dilution and wash buffer from 5X stock in mill-Q water.
  2) Mix the **Benzonase Nuclease** treated filtrate with equal volume of 1X dilution buffer.

Adenovirus Amplification

1) Grow 293 cells in 10x150mm plates for 4 days before infection (100% confluent).
2) Infect the 293 cells in 2% media.
3) Incubate the cells at 37˚C until Cytopathic effects are complete till cells starts floating.
4) Harvest cells, centrifuge at 3000-RPM, store the cell pellet and supernatant separately.
5) Resuspend the cell pellet in 5 mL of PBS. Lyse cells with 4-5 freeze cycles. Centrifuge and transfer the CVL to a sterile tube.
6) Store the CVL and supernatant in –80C until further use.

Filter Purification (Removal of Cell Debris)

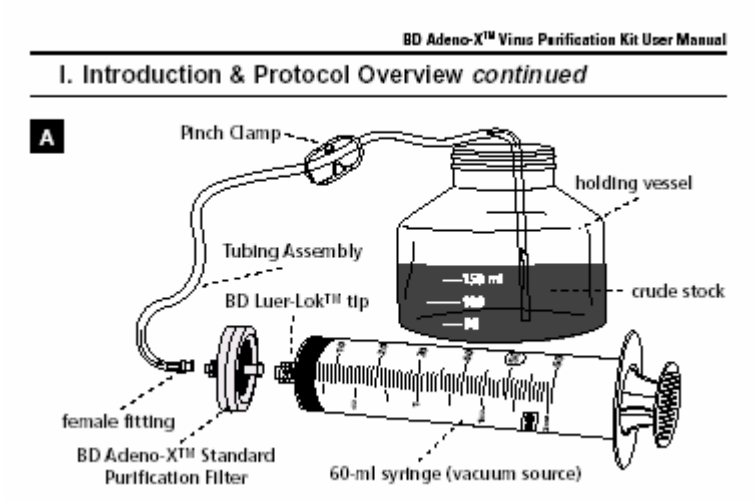
Carry out most of the following steps in BL-2 Hood unless indicated otherwise.

1) Pool the supernatant and CVL.

2) Unwrap a bottle –top filter (from the Kit package) and place in the biosafety hood. Remove the lid from the top of the unit and carefully place the pre-filter disc on top of the 0.45-micron filter.
3) Connect the bottle-top filter to a vacuum source.
4) With the vacuum off, add 10 mL of 5% media to the pre filter so that it adheres uniformLy to the 0.45-micron filter below it (Be careful not to dislodge the pre-filter).
5) With vacuum off, carefully pour the pooled supernatant into the top of the filter unit. Being careful to not overfill.
6) Turn the vacuum on. When the bottom collection vessel becomes full, disconnect the vacuum line and unscrew the collection vessel.
7) Save the filtrate in a sterile flask
8) To remove any contaminating cellular DNA, add **Benzonase Nuclease** (Novagen) to the filtrate. The final concentration should be 10 units Benzonase per mL of the filtrate and incubate at 37˚C for 30 min.

Purification:

1) Collect the following components and place them within easy reach.
   - Tubing assembly and BD adeno X purification filter.
   - 1X wash buffer
   - 1X dilution buffer
   - Sterile PBS
   - 5,30 mL syringes
   - Container for holding biohazardous liquid waste.
2) Connect the BD adeno X purification filter and tubing assembly as shown in Figure below.



3) Do not place the tubing in the virus-containing solution yet.

4) Remove air from the filter and tubing assembly by placing the tube in 50 mL tube containing PBS. Fill a 10 mL syringe with 5-10 mL of sterile PBS.
5) Attach the syringe to the filter and push the PBS through the assembly. Place the tubing line in the virus containing solution.
6) Pull the virus containing solution through filter at ~20 mL/min. Pull the solution through the filter with the attached 60 mL syringe. When the syringe is full, clamp the tubing assembly, disconnect the syringe, and empty the syringe into a biohazard waste container. Reattach the syringe, unclamp the line, and continue pulling the solution through the filter.
7) Repeat this steps until the entire volume has been filtered. Avoid drawing air into the system.
8) Wash the adenovirus collected on the membrane by transferring the tube assembly from the virus solution to the 1X wash buffer container.
9) Pull the entire volume of the wash buffer through the filter at 20min/mL slowly as described above. Replace the 60 mL syringe with the 3 mL syringe containing elution buffer.

Virus Elution

1) Fill a 3 mL syringe with 1X elution buffer. Connect the syringe as described above [to the female port on the filter cartridge,] and remove the tube assembly from the other side.
2) Push 1mL of elution buffer through the filter into a sterile 15mL conical centrifuge tube.
3) Incubate the filter at room temperature for 5 min , then push the remaining elution buffer through to collect the remaining adenovirus.
4) Use air to push any remaining virus from the filter.

Virus Estimation

1) Use air to push any remaining virus from the filter.
2) Use elution buffer for blank. Make sure the cuvette is clean outside without any fingerprints.
3) Read O.D at 260nm. Record OD reading. Estimate the amount of virus. (Virus (pfu per mL) = OD at 260 nm x5 x10X10E10 pfu/mL)).
4) Add equal volume of dialysis buffer and store at –70C.
5) Determine the adenoviral titer by BD Adeno-X rapid titer kit.